Soluble Urokinase Plasminogen Activator Receptor (suPAR) in the Diagnosis of Pulmonary Embolism

Pulmoner Emboli Tanısında Soluble Urokinase Plasminogen Activator Receptor (suPAR)

Pulmonary Embolism and suPAR

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Özet

Amaç: Çalışmamızın amacı, sağlıklı kişiler ve pulmoner emboli (PE)' li hastalarda plazma soluble urokinase plasminogen activator receptor (suPAR) düzeylerini karşılaştırmak ve ayrıca PE tanısında suPAR' ın değerini araştırmaktır. Gereç ve Yöntem: Spiral bilgisayarlı tomografi pulmoner anjiyografi kullanılarak akut PE tanısı alan otuz hasta çalışmaya dahil edildi. suPAR ve D-dimer düzeyleri tanı anında ölçüldü. Yirmi dokuz yaş-cinsiyet uyumlu sağlıklı birey çalışmaya seçildi. Plazma örneklerinde suPAR düzeyini kantitatif belirlemek için suPARnostic ELISA Standard Kit (ViroGates A/S, Birkerød, Denmark, Code No. A001) kullanıldı. Bulgular: Ortanca (%95 CI) suPAR düzeyi kontrol grubunda 3.3 (2.9-4.2) ng/mL, PE grubunda 6.4 (6.4-10.5) ng/mL ölçüldü (P<0.001, Şekil 1). PE hastalarında suPAR seviyesi anlamlı olarak yüksekti (P<0.001). ROC curve analizinde, eğri altında kalan alan 0.871 (CI; 0.776-0.965), suPAR cut-off değerinde spesifite ve sensitivitesi sırasıyla %83 ve %82 bulundu. Yüksek suPAR'a (⊠4.3 ng/ml) sahip PE hastalarında hastanede kalış, düşük olanlara göre anlamlı daha uzundu (p=0.049). D-dimer ve suPAR arasında istatiksel anlamlı pozitif korelasyon vardı (r=0.530, P=0.004). Tartışma: Bu çalışma, PE tanısı için suPAR düzeylerinin iyi sensitivite ve spesifiteli bir belirteç olabileceğini gösterdi. Ancak, pulmoner embolide suPAR' ın tanısal ve prognostik önemini önemini göstermek için büyük prospektif çalışmalara ihtiyaç vardır.

Anahtar Kelimeler

Pulmoner Emboli; suPAR; Tanı

Abstract

Aim: The goal of our study was to compare the suPAR levels between pulmonary embolism (PE) patients and healthy subjects and also to investigate the value of suPAR in the diagnosis of PE. Material and Method: Thirty patients diagnosed with acute PE using spiral computerized tomographic pulmonary angiography were included in the study. suPAR and D-dimer levels were measured at the time of diagnosis. Twenty-nine age- and sex-matched healthy subjects were chosen for the study. The suPARnostic ELISA Standard Kit (ViroGates A/S, Birkerød, Denmark, Code No. A001) was used for the quantitative determination of suPAR levels in plasma samples. Results: Median (95% CI) suPAR level measured in the PE group was 6.4 (6.4-10.5) ng/mL, compared to 3.3 (2.9-4.2) ng/mL in the control group (P<0.001). suPAR levels were significantly higher in the patients with PE than in controls (P<0.001). Receiver operator characteristic (ROC) curve analysis was found as 0.871 [confidence interval (CI) 0.776-0.965] area under the curve, 83% specificity, and 82% sensitivity at the cut-off of suPAR. Patients with higher suPAR (4.3 ng/ml) had significantly longer hospital stays than patients with lower suPAR (p=0.049). There was a statistically significant positive correlation between D-dimer and suPAR (r=0.530, P=0.004). Discussion: This study suggests that suPAR may be a biomarker with good sensitivity and specificity for diagnosis of PE. However, large prospective studies are required to demonstrate the diagnostic and prognostic significance of suPAR in PE.

Keywords

Pulmonary Embolism; suPAR; Diagnosis

DOI: 10.4328/JCAM.4557 Received: 15.04.2016 Accepted: 27.04.2016 Printed: 01.01.2017 J Clin Anal Med 2017;8(1): 6-9
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Introduction

Pulmonary embolism (PE) is a common and potentially fatal condition. Most patients who die from pulmonary embolism do so within the first few hours without accurate diagnosis. Patients with PE often have nonspecific symptoms and the diagnosis is often delayed [1]. Earlier diagnosis of PE may decrease the morbidity and mortality. There are still difficulties in the diagnosis of PE [2]. Noninvasive diagnostic tests such as plasma D-dimer measurement, lower limb deep vein compression ultrasonography (CUS), ventilation-perfusion scintigraphy (V/Q scan), and chest multi detector computed tomography pulmonary angiography (CTPA) have been used for the diagnosis of PE [3]. Plasma D-dimer measurement, a noninvasive diagnostic tool, is frequently used. It is a degradation product produced by plasmin-mediated proteases of cross-linked fibrin. It is the most reliable tool for excluding pulmonary embolism in younger patients who have no associated comorbidity or history of venous thromboembolism and whose symptoms are of short duration [4]. Advanced age, pregnancy, active malignancy, recent surgical interventions, liver failure, rheumatoid arthritis, and the presence of infection may result in false positive plasma D-dimer measurement. Anti-coagulation therapy, the presence of small clots, isolated small pulmonary infarcts, and existing symptoms persisting for more than 5 days may lead to false-negative plasma D-dimer measurement results [5]. Therefore, new tests should be identified in order to exclude PE and to reduce the number of these advanced imaging tests performed. The urokinase plasminogen activator receptor (uPAR) is a three domain membrane bound receptor, mainly expressed on immunologically active cells (e.g. neutrophils, activated T cells, macrophages) and vascular endothelial cells [6, 7]. The soluble form of uPAR (suPAR) can be generated when uPAR is cleaved from the surface of such cells during inflammatory stimulation. It is linked to cellular and vascular inflammatory processes [8]. With its ligand, urokinase plasminogen activator (uPA), it participates in numerous immunologic functions including migration, adhesion, angiogenesis, fibrinolysis, and cell proliferation [9]. SuPAR is a measurable protein in the circulating blood of all individuals. It has been tested as a prognostic and diagnostic tool in a number of infectious and inflammatory conditions. In contrast to most pro-inflammatory and acute response biomarkers, circadian changes in plasma suPAR are minimal and the in vitro stability of suPAR is high [10, 11]. Numerous observational studies have shown increased suPAR in patients with cancer and various infectious and inflammatory diseases, including infections with human immunodeficiency virus (HIV), malaria, tuberculosis, central nervous system infections, arthritis, liver fibrosis, and inflammatory bowel disease [12]. Recent studies have pointed to its association with atherosclerosis and the development of cardiovascular disease (CVD) [13, 14].

In the Malmö Diet and Cancer Study population, one study reported that development of venous thromboembolism was higher in individuals with higher suPAR levels [15]. However, less is known about the relationship between suPAR and PE.

The goal of this study was to compare the suPAR levels between PE patients and healthy people and to investigate the value of suPAR in the diagnosis and prognosis of PE.

Material and Method

Study Design

Thirty patients hospitalized and monitored with a PE diagnosis in the Chest Disease Clinic of Suleyman Demirel University Hospital were enrolled in the study. The diagnosis of pulmonary thromboembolism was made based on its compatibility with a filling defect of PTE on spiral computerized tomographic pulmonary angiography according to a predefined standard protocol. The local ethics committee approved the present study. Patients with hypertension, diabetes mellitus, chronic renal failure, liver disease, heart failure, acute or chronic infection, accompanying autoimmune disease, and malignancy were excluded. 29 consecutive sex- and age-matched healthy individuals without relevant current status and medical history were included. The exclusion criteria in the control group were the same as those in the patient group. In patients, plasma D-dimer examinations were performed usig a automatic coagulation analyzer and the immune turbidimetry method, with reference values of 69–243 ng/ml.

Plasma suPAR Measurement

Whole EDTA-blood samples were centrifuged at 3,000 x g for 10 minutes. The plasma samples were transferred in separate tubes and stored at 80°C. The suPARnostic ELISA Standard Kit (ViroGates A/S, Birkerød, Denmark, Code No. A001) was used for the quantitative determination of suPAR levels in plasma samples. The levels of plasma suPAR were determined according to the manufacturer's instructions. The absorbance of samples was measured at 450 nm using an Organon Teknika 530 Microplate Reader. The suPAR concentrations of each specimen were determined by interpolation on the standard curve that was created by the absorbance of the standards. The suPAR concentrations of each specimen were expressed as ng/mL. The detection limit of the assay was estimated to be 0.1 ng/mL.

Statistical analysis

Statistical analysis was performed using SPSS 15.0. Normal distribution of data was analyzed using the Kolmogorov-Smirnov test, and the Mann-Whitney U-test was used to compare the groups. Statistical significance was set at $p < 0.05$. In addition, receiver operating characteristic (ROC) curve analysis was performed.

Results

Fifty nine individuals (30 patients with PE and 29 healthy subjects) were enrolled in the study. Demographic characteristics (age and sex) in the PE and control groups were similar. There was a total of 30 patients, of whom 14 (46.7%) were female and 16 (53.3%) were male. The mean age of the patients was 61.73 (±17.4) years. The PE group's clinical characteristics are shown in Table 1. The median (95% CI) suPAR level measured in the PE group was 6.4 (6.4-10.5) ng/mL, compared to 3.3 (2.9-4.2) ng/mL in the control group (P<0.001, Figure 1). suPAR levels were significantly higher in the patients with PE (P<0.001). Receiver operating characteristic (ROC) curve analysis was performed to determine cutoff thresholds in discriminating between PE and control group plasma suPAR levels. The area under the ROC for that purpose was 0.871 (95% CI; 0.776-0.965). A suPAR cutoff

Table 1. PE group's demographic and clinical characteristics

Charateristic	n (%) or Mean (±SD)
Mean age (year)	61.73 (±17.4)
Sex	
Female	14 (46.7)
Male	16 (53.3)
Sign and symptoms	
Dyspnea	26 (86.7)
Chest pain	20 (66.7)
Syncope	6 (20.0)
Hemoptysis	5 (16.7)
DVT symptoms	3 (10.0)
Physical examination	
Heart rate (beats/min)	87 (13.0)
Systolic BP (mmHg)	118.6 (21.6)
Diastolic BP (mmHg)	72.6 (10.8)
Surgery or trauma history	11 (36.6)
DVT	9 (33.3)
Thrombus location	
Main pulmonary artery (right or left)	15 (50)
Lobar pulmonary arteries (right or left)	22 (73.3)
Segmental and subsegmental pulmonary arteries (right or left)	16 (53.3)
Pulmonary artery pressure (mmHg, ECHO)	39.8 (17.4)
Wells score	4.6 (±1.10)
D-dimer leves (ng/ml)	3060 (±4235)

point in patients with PE > 4.3 ng/mL had specificity and sensitivity of 83% and 82%, respectively (Figure 2). Patients with higher suPAR (4.3 ng/ml) had significantly longer hospital stays than patients with lower suPAR. The median days of hospital stay was 8 in patients with higher suPAR levels and 6 in patients with lower suPAR levels. This difference was statistically significant (p=0.049). There was a statistically significant positive correlation between D-dimer and suPAR (r=0.530, P=0.004).

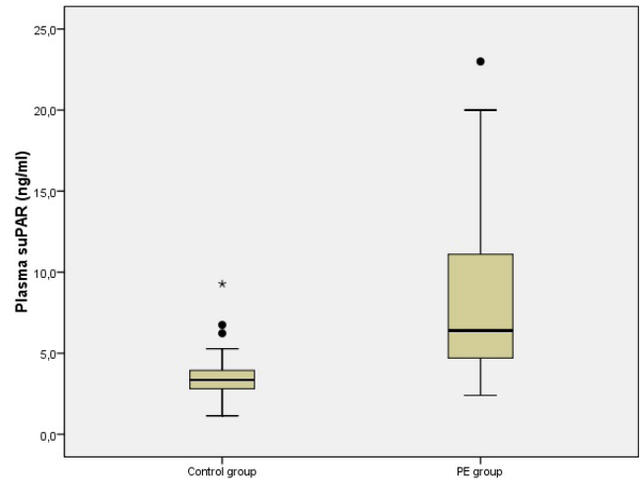

Figure 1. Plasma suPAR levels in the PE and control groups. Horizontal lines represent the median of suPAR levels for PE and control groups, 6.4 and 3.3 ng/mL, respectively.

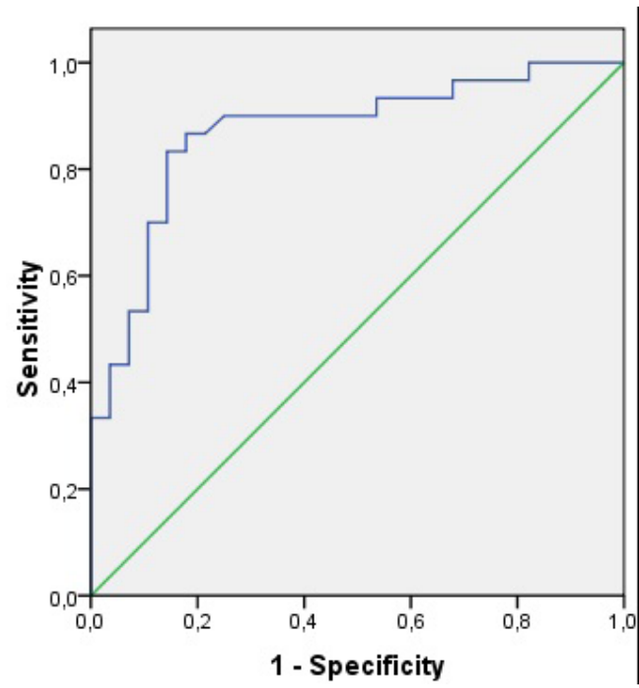

Figure 2. Receiver operator characteristic curve showing specificity and sensitivity percentages of suPAR in PE patients. Area under the curve 0.871, suPAR cut-off value 4.3ng/ml, sensitivity 82%, specificity 83%.

Discussion

The present study has novel findings: serum suPAR levels were significantly higher in patients with PTE than in healthy controls. There was a statistically significant positive correlation between D-dimer and suPAR. In addition, patients with higher suPAR levels had significantly longer hospital stays than patients with lower suPAR levels.

The suPAR level indicates both an active systemic inflammation and a low grade inflammation [16]. It has been suggested that suPAR is involved in the plasminogen-activating pathway, inflammation and modulation of cell adhesion, migration, and proliferation [7]. Systemic levels of suPAR were significantly higher in critically ill patients compared to healthy controls [11, 17]. suPAR has been linked to endothelium dysfunction, damaged cardiac microcirculation, increased vascular stiffness, and finally, more extensive atherosclerosis [18]. Elevated levels of suPAR were associated with increased risk of future CVD independently for traditional risk factors and subclinical organ damage [19]. Several longitudinal population-based studies have reported an association between plasma suPAR levels and increased risk of CVD [13, 20].

Endothelial damage is one of the causes of thrombus formation in PE. suPAR was found to be positively correlated with the number of neutrophils, leucocytes, monocytes, and eosinophils. This supports suPAR as a marker associated with inflammation. In our study, suPAR levels were significantly higher in the patients with PE compared to healthy controls. These results may be due to the fact that suPAR is produced from endothelial cells and has an important role in the fibrinolytic system [7, 21]. Several studies have investigated the prognostic value of suPAR. High systemic levels of suPAR were associated with the need for ICU admission [11]. Studies involving patients with myocardial ischemia (acute coronary syndrome or MI) have demonstrated that suPAR is associated with mortality [20, 22]. One study involving 449 patients with acute chest pain and suspected non-ST-elevation acute coronary syndrome demonstrated suPAR to be a strong and independent marker of all causes of mortality over the mid- and long-term [23]. We did not find an association between suPAR levels and mortality.

Plasma D-dimer measurement is commonly used as the first test in patients suspected of having acute PE. Several factors other than PE or deep vein thrombosis (DVT) are associated with positive D-dimer results. The positive predictive value of D-dimer with advanced age, malignancy, pregnancy, heart failure, pneumonia, sepsis, and kidney failure is quite low [2,24]. Specificity is reported to be between 40% and 60% [25]. A suPAR cutoff point > 4.3 ng/mL showed 83% specificity and 82% sensitivity in patients with PE. Also, D-dimer levels were significantly correlated with suPAR levels.

The present study has several limitations. First, the study involves a relatively small number of patients and controls. Second, we used a cross-sectional design for this study. Third, the large number of exclusion criteria may also be a limitation of this study, decreasing the utility of this test as an effective adjunct to guide the diagnosis of PE relative to other conditions that also may alter suPAR levels.

Consequently, we suggest that plasma suPAR may be a biomarker with good sensitivity and specificity for diagnosis of PE. However, further prospective studies with a larger population are required to demonstrate the diagnostic and prognostic significance of suPAR.

Competing interests

The authors declare that they have no competing interests.

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How to cite this article:

Dirican N, Aynali A, Cakir M, Atalay S, Bircan HA, Ozturk O, Akkaya A. Soluble Urokinase Plasminogen Activator Receptor (suPAR) in the Diagnosis of Pulmonary Embolism. J Clin Anal Med 2017;8(1): 6-9.